IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2325
de la MONTE *et al.*	Art Unit: 1635
Appl. No. 09/380,203	Examiner: Whiteman, B.
§ 371 date: April 25, 2000	Atty. Docket: 0609.4370001/RWE/FRC
For: **Transgenic Animals and Cell Lines for Screening Drugs Effective for the Treatment or Prevention of Alzheimer's Disease**	

Brief on Appeal Under 37 C.F.R. § 1.192

Mail Stop Appeal Brief - Patents

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

A Notice of Appeal from the final rejection of claims 1-3, 5, 6, 10-13, 35 and 44-47 was filed on December 15, 2003. Appellants hereby file this Appeal Brief in triplicate, together with the required brief filing fee.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

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I. Real Parties in Interest (37 C.F.R. § 1.192(c)(1))

The real parties in interest in this appeal are The General Hospital Corporation and Nymox Corporation.

II. Related Appeals and Interferences (37 C.F.R. § 1.192(c)(2))

Appellants have filed a Notice of Appeal and an Appeal Brief in U.S. Patent Application No. 09/964,678, which is a divisional of the above-captioned patent application.

III. Status of Claims (37 C.F.R. § 1.192(c)(3))

The above-captioned application was filed on August 26, 1999 under 35 U.S.C. § 371 as the U.S. National Phase of International Patent Application No. PCT/US98/03685. The date of receipt of 35 U.S.C. § 371 requirements for this application is April 25, 2000.

As originally filed, this application contained a total of 34 claims.

In an Amendment filed on January 16, 2002, claims 7-9 and 14-34 were cancelled, claims 1, 3, 4, 10 and 11 were amended, and claims 35-38 were added.

In an Amendment filed on July 9, 2002, claim 4 was cancelled, claims 1, 6, 36, 37 and 38 were amended, and claims 39-49 were added.

In an Amendment filed on January 21, 2003, claims 1, 10 and 44 were amended.

In an Amendment filed on July 7, 2003, claims 38 and 48 were cancelled, and claims 1, 10, 39, 44, 45 and 49 were amended.

Claims 1-3, 5, 6, 10-13, 35-37, 39-47 and 49 are pending in this application. Claims 39-43 and 49 are allowed. Claims 36 and 37 are objected to. Claims 1-3, 5, 6, 10-13, 35 and 44-47 are now on appeal. A copy of the claims on appeal, the objected claims and the allowed claims can be found in the attached Appendix (section X, below).

IV. Status of Amendments (37 C.F.R. § 1.192(c)(4))

All amendments have been entered. No amendments have been filed subsequent to the issuance of the final Office Action dated September 15, 2003.

V. Summary of Invention (37 C.F.R. § 1.192(c)(5))

Alzheimer's disease ("AD") is a neurodegenerative disease characterized by prominent atrophy of corticolimbic structures with neuronal loss, neurofibrillary tangle formation, aberrant proliferation of neurites, senile plaques and βA4-amyloid deposition in the brain. *See* specification at page 2, lines 1-4. In previous studies, the inventors demonstrated that polyclonal antisera prepared against a pancreatic protein had increased immunoreactivity in the brains of AD patients. *See* specification at page 5, lines 3-9. Using the polyclonal antisera, a cDNA clone corresponding to the antigen recognized by the antisera was isolated from an AD brain expression library. *See* specification at page 5, lines 9-11. The polypeptide encoded by the isolated cDNA was designated "AD7c-NTP" (NTP = neuronal thread protein). *See* specification at page 17, lines 17-18. AD7c-NTP is expressed in neurons and is over-expressed in brains of AD patients. *See id.* Abnormal expression of AD7c-NTP is a phenotype associated with AD. *See* specification at page 18, lines 7-9.

The nucleotide sequence of the AD7c-NTP gene is set forth in the application as SEQ ID NO:1. The amino acid sequence of AD7c-NTP is set forth in the application as SEQ ID NO:2. *See* specification at page 7, lines 16-17, and Figs. 1A-1C.

Claim 1 is directed to a DNA construct which comprises the DNA molecule of SEQ ID NO:1 or a DNA molecule which is at least 90% homologous thereto, wherein the DNA molecule is under control of a heterologous neuro-specific promoter, and wherein the DNA molecule codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells. Claims 2 and 3 depend from claim 1 and specify that the DNA construct is contained within a vector or a virion, respectively. Claim 5 is directed to a host cell transformed with the DNA construct of claim 1. Claim 6 depends from claim 5 and specifies that the host cell is a neuronal cell. Claim 35 depends from claim 1 and specifies that the activity of AD7c-NTP is selected from the group consisting of neuritic sprouting, nerve cell death, nerve cell degeneration, neurofibrillary tangles, and irregular swollen neurites. Support for claims 1, 2, 3, 5, 6 and 35 can be found throughout the specification, for example, at page 18, line 15, through page 19, line 30.

Claims 10 and 44 are directed to *in vitro* methods for screening candidate drugs that are potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, or glioblastomas. The methods of claims 10 and 44 comprise: (a) contacting a candidate drug with the host cell of claim 5 or 42, respectively, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct of the host cell; (ii) the increased degradation of the protein coded for by the DNA construct of the host cell; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell

death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host cell, wherein the host cell is a neuronal cell. The claims specify that (i), (ii) or (iii) are due to the drug candidate compared to a control cell line which has not contacted the candidate drug. Claim 11 depends from claim 10 and specifies that the protein has SEQ ID NO:2. Claim 45 depends from claim 44 and specifies that the DNA molecule comprises a DNA sequence having the nucleotide sequence set forth in SEQ ID NO:1. Claims 12 and 46 depend from claims 10 and 44, respectively, and specify that the protein is over-expressed by the host cell. Claims 13 and 47 depend from claims 10 and 44, respectively, and specify that the cell is a neuronal cell. Support for claims 10-13 and 44-47 can be found throughout the specification, for example, at page 21, line 3, through page 24, line 9.

VI. Issues on Appeal (37 C.F.R. § 1.192(c)(6))

A. Written Description

The first issue on appeal is whether claims 1, 2, 3, 5, 6, 10-13 and 35 are unpatentable under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. *See* Paper No. 32, page 3.

B. Enablement

The second issue on appeal is whether claims 1, 2, 3, 5, 6, 10-13, 35 and 44-47 are unpatentable under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. *See* Paper No. 32, page 6.

VII. Grouping of Claims (37 C.F.R. § 1.192(c)(7))

For the purpose of this appeal, the claims do not stand or fall together. The claims are grouped as follows:

Group 1. Claims 1, 2, 3, 5, 6 and 35 (encompassing DNA constructs comprising a DNA molecule that is at least 90% homologous to SEQ ID NO:1, and host cells transformed thereby);

Group 2. Claims 10, 12 and 13 (encompassing screening methods that include the use of a host cell transformed with a DNA construct comprising a DNA molecule that is at least 90% homologous to SEQ ID NO:1);

Group 3. Claims 11, 44, 46 and 47 (encompassing screening methods that include the use of a host cell transformed with a DNA construct comprising a DNA molecule that is specified to encode a protein having SEQ ID NO:2); and

Group 4. Claim 45 (encompassing screening methods that include the use of a host cell transformed with a DNA construct comprising a DNA molecule that is specified to have SEQ ID NO:1).

An explanation for the grouping of the claims is provided in the Argument section, immediately below.

VIII. Argument (37 C.F.R. § 1.192(c)(8))

A. Explanation for the Grouping of the Claims

1. Grouping of the Claims on the Issue of Written Description

The written description rejection is based on the Examiner's contention that DNA molecules that are at least 90% homologous to SEQ ID NO:1 are not adequately described in the specification. *See* Paper No. 32, pages 3-5. The Examiner stated, however, that "[t]he specification provides sufficient description of SEQ ID NO: 1 and a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 2." *See* Paper No. 32, page 3. Claims 11, 44, 46 and 47 (Group 3), and claim 45 (Group 4) encompass screening methods that include the use of a host cell transformed with a DNA construct comprising a DNA molecule that is specified to either encode a protein having SEQ ID NO:2, or to have SEQ ID NO:1. Thus, the rejection for alleged insufficient description does not apply to the claims of Groups 3 or 4.

2. *Grouping of the Claims on the Issue of Enablement*

The Examiner has set forth two separate bases for the enablement rejection. First, the Examiner asserted that it would have required undue experimentation for a person of ordinary skill in the art to obtain a DNA molecule that is at least 90% homologous to SEQ ID NO:1 and that codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells. *See* Paper No. 32, pages 6-8. The Examiner stated, however, that "the specification is enabling only for a DNA construct, which comprises the DNA molecule of SEQ ID NO: 1 or a DNA molecule comprising a nucleotides [sic] sequence encoding the amino acid sequence set forth in SEQ ID NO: 2." Paper No. 32, page 6.

The second basis for the enablement rejection is the Examiner's assertion that the specification does not enable the practice of the claimed methods. *See* Paper No. 32, pages 8-10.

Claims 11, 44, 46 and 47 (Group 3), and claim 45 (Group 4) encompass screening methods that include the use of a host cell transformed with a DNA construct comprising a DNA molecule that is specified to either encode a protein having SEQ ID NO:2, or to have SEQ ID NO:1. Thus, the Examiner's first basis for the enablement rejection does not apply to the claims of Groups 3 or 4.

Claims 1, 2, 3, 5, 6 and 35 (Group 1) are not directed to methods. Thus, the Examiner's second basis for the enablement rejection does not apply to the claims of Group 1.

B. Written Description

1. The Written Description Rejection

Claims 1, 2, 3, 5, 6, 10-13 and 35 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. *See* Paper No. 32, page 3.

The Examiner acknowledged that the specification adequately describes DNA molecules having SEQ ID NO:1 and DNA molecules that encode a protein having the amino acid sequence of SEQ ID NO:2. *See* Paper No. 32, page 3. However, according to the Examiner, "[t]he specification does not provide sufficient description of a genus of DNA molecules with 90% homology to SEQ ID NO: 1 that codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells." Paper No. 32, page 4.

As discussed below, the specification provides more than adequate description for the DNA molecules that are encompassed by or included within the subject matter of the claims. In addition, the Examiner has not established a *prima facie* case of insufficient written description. Therefore, the written description rejection was improper and should not be sustained.

2. Legal Standard for Written Description

To satisfy the written description requirement of 35 USC § 112, first paragraph, an Applicant must convey with reasonable clarity to those skilled in the art that, as of the

effective filing date, the Applicant was in possession of the invention. *See Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). As made clear by the Federal Circuit, "[t]he written description requirement does not require the applicant 'to describe exactly the subject matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed.'" *Union Oil Co. of Cal. v. Atlantic Richfield Co.*, 208 F.3d 989, 997, 54 USPQ2d 1227, 1232 (Fed. Cir. 2000); *see also Amgen Inc. v. Hoechst Marion Roussel Inc.*, 65 USPQ2d 1385, 1397 (Fed. Cir. 2003) ("[t]he purpose of the written description requirement is to prevent an applicant from later asserting that he invented that which he did not.")

The Federal Circuit has recently adopted the standard for determining compliance with the written description requirement as set forth in the USPTO's "Guidelines for the Examination of Patent Applications under 35 U.S.C. § 112, first paragraph, Written Description Requirement." *See Enzo Biochem, Inc. v. Gen-Probe Inc.*, 296 F.3d 1316, 1324, 63 USPQ2d 1609, 1613 (Fed. Cir. 2002). According to the USPTO's Guidelines:

> An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention, i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.

MPEP § 2163; *See also, Enzo*, 296 F.3d at 1324, 63 USPQ2d at 1613.

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the Examiner to rebut the presumption. *See In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA

1971). The Examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person of ordinary skill in the art would not recognize in an Applicant's disclosure a description of the invention defined by the claims. *See In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976); *see also* MPEP § 2163.04.

3. The Specification Provides More Than Adequate Written Description for DNA Molecules Which are at Least 90% Homologous to SEQ ID NO:1 and that Code For Proteins Having an Activity of AD7c-NTP When Overexpressed in Neuronal Cells

(a) In View of the Specification, a Person of Ordinary Skill in the Art Would Conclude that Appellants had Possession of the Claimed Subject Matter

A person of ordinary skill in the art, upon reading the specification, would conclude that, as of the effective filing date, Appellants had invented the claimed subject matter. First, the specification provides detailed information on the isolation of the AD7c-NTP cDNA. *See* specification at page 33, line 8, through page 34, line 4. The specification sets forth the complete nucleotide sequence of the AD7c-NTP cDNA (SEQ ID NO:1). The specification also describes and illustrates the sequence characteristics and motifs found in the amino acid sequence encoded by SEQ ID NO:1. *See* specification at page 34, line 5, through page 35, line 28; *see also* specification at page 7, line 21, through page 8, line 3 and Figure 1.

Moreover, the specification clearly indicates that the invention includes DNA molecules that are at least 90% homologous to SEQ ID NO: 1. *See* specification at page 18, lines 15-18. The specification also teaches how DNA molecules that are at least 90% homologous to SEQ ID NO:1 can be obtained, and how to determine if a DNA molecule that is at least 90% homologous to SEQ ID NO:1 codes for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells. *See* specification at page 19, line 3, through page 24, line 9. The specification also provides a working example that describes and illustrates the neuronal abnormalities that are caused by over-expressing AD7c-NTP in neuronal cells. *See* specification at page 46, lines 1-26 and Figs. 6A-6G. (The ability of persons of ordinary skill in the art to obtain the DNA molecules of the invention without undue experimentation is discussed in detain in section VIII.C.3, below.)

The detail provided in the specification for obtaining DNA molecules that are at least 90% homologous to SEQ ID NO: 1 and for determining whether they encode proteins having an activity of AD7c-NTP when overexpressed in neuronal cells would indicate to persons of ordinary skill in the art that Appellants were in possession of DNA molecules having a nucleotide sequence that is at least 90% identical to SEQ ID NO: 1.

(b) The USPTO's Guidelines Support the Conclusion that the Written Description Requirement is Fully Satisfied for the Claimed Subject Matter

The USPTO's Synopsis of Application of Written Description Guidelines (hereinafter "Written Description Synopsis") specifically supports Appellants' assertion

that the written description requirement is satisfied for the DNA molecules of the invention.

At the outset, Appellants acknowledge that the precise details of the examples in the Written Description Synopsis do not exactly match the details of the present claims. Indeed, it is highly unlikely that any "real life" situation would precisely match every detail of the examples in the Written Description Synopsis. The examples are presented in general terms and are intended to illustrate the *analytical process* that the USPTO believes should be used to assess compliance with the written description requirement. The examples do not set any absolute rules or numerical cut-offs. When the analytical process that is illustrated in the Written Description Synopsis is applied to the circumstances surrounding the present claims, it must be concluded that the written description requirement is fully satisfied.

Example 14 of the Written Description Synopsis (copy attached hereto as Exhibit 1) involves an analysis of the following claim: "A protein having SEQ ID NO:3 and variants thereof that are at least 95% identical to SEQ ID NO:3 and catalyze the reaction of A→B." Written Description Synopsis at page 53. The specification supporting this claim provides the following information:

> The specification exemplifies a protein isolated from liver that catalyzes the reaction of A→B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO:3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Written Description Synopsis at page 53.

The Written Description Synopsis, Example 14, concludes that the disclosure meets the requirements of 35 USC § 112, first paragraph, in part because "procedures for making variants of SEQ ID NO:3 which have 95% identity to SEQ ID NO:3 and retain its activity are conventional in the art." *See id.* Moreover, it is noted that:

> [t]he single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO:3 which are capable of the specified catalytic activity. One of skill in the art would conclude that applicant was in possession of the necessary common attributes possessed by the members of the genus.

Written Description Synopsis at pages 54-55.

The situation presented in Example 14 of the Written Description Synopsis closely parallels the circumstances surrounding the DNA molecules that are encompassed by or are included within the subject matter of Appellants' claims and the written description provided therefor. As such, Appellants submit that the guidance and instructions provided by the USPTO for analyzing a claim for compliance with the written description requirement strongly supports Appellants' assertion that the written description requirement of § 112, first paragraph, is satisfied for Appellants' claims.

First, in Example 14, it is stated that "all variants [encompassed by the claim] must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO:3." Written Description Synopsis at page 54. Similarly, all of the species of DNA molecules encompassed by or included within the subject matter of the claims on

appeal must have at least 90% homology to SEQ ID NO:1 and must code for a protein having an activity of AD7c-NTP when over-expressed in neuronal cells.

Second, it is noted in Example 14 that "[t]here is a single species disclosed, that species being SEQ ID NO:3," and that "[t]here is actual reduction to practice of the single disclosed species." Written Description Synopsis at page 54. Likewise, Appellants have disclosed SEQ ID NO:1 in the specification and have shown actual reduction to practice of SEQ ID NO:1. *See* specification at page 33, line 9, through page 35, line 28 (describing the isolation of the AD7c-NTP cDNA and the characteristics of the molecule); *see also* Figs. 1A-1C.

Third, according to Example 14, "procedures for making variants of SEQ ID NO:3 which have 95% identity to SEQ ID NO:3 and retain its activity are conventional in the art." Written Description Synopsis at page 53. Likewise, procedures for making DNA molecules which are at least 90% homologous to SEQ ID NO:1 and which encode proteins that retain the activity of AD7c-NTP are conventional in the art. As stated in the specification, DNA molecules which are at least 90% homologous to SEQ ID NO:1 may be isolated from cDNA libraries of humans and animals by hybridization under stringent conditions to the DNA molecule of SEQ ID NO:1 according to methods known to those of skill in the art. *See* specification at page 19, lines 3-15. Appellants note that many other methods for obtaining DNA molecules that are included within the presently claimed invention were well known to persons having ordinary skill in the art at the time of the invention; examples include random and directed mutagenesis of a DNA molecule to produce a variant of SEQ ID NO:1 that is at least 90% homologous thereto. *See* section VIII.C.3(a), below. In addition, proteins encoded by variants of SEQ ID NO:1

can easily be tested for AD7c-NTP activity using the procedures described in the specification, as well as with other methods that were conventional in the art for testing the biological activity of a protein. *See* section VIII.C.3(b), below.

Fourth, in Example 14 of the Written Description Synopsis, it is stated that "an assay is described [in the specification] which will identify other proteins having the claimed catalytic activity." Written Description Synopsis at page 53. Correspondingly, in Appellants' specification, assays are described which will identify other DNA molecules encoding proteins having an activity of AD7c-NTP. For example, the specification describes the production of transgenic animals which over-express AD7c-NTP and the analysis of such animals for "evidence of neuronal or neuritic abnormalities associated with Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas and gliblastomas." *See* specification at page 20, lines 1-29. The specification also provides a working example of an *in vitro* assay for AD7c-NTP activity involving the over-expression of AD7c-NTP in neuronal cells and the analysis of such cells for growth properties and morphology, including the occurrence of apoptosis and neuritic sprouting. *See* specification at page 45, line 16, through page 46, line 26, and Figs. 6A-6G.

As demonstrated above, the hypothetical situation described in Example 14 of the USPTO's Written Description Synopsis is very similar to the situation presented for the DNA molecules encompassed by or included within the subject matter of the claims on appeal. Since the USPTO guidelines conclude that adequate written description is provided for the hypothetical claim in Example 14, it follows that there is adequate written description for the subject matter of the claims on appeal.

In *Enzo*, the Federal Circuit also made specific reference to Example 9 of the Written Description Synopsis. *See Enzo*, 296 F.3d at 1328, 63 USPQ2d at 1615. The analysis set forth in Example 9 (copy attached hereto as Exhibit 2) further supports Appellants' contention that the written description requirement is satisfied for the claims involved in this appeal and that the rejection was in error. Example 9 involves an analysis of the written description provided for a claim to "[a]n isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1, wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity." Written Description Synopsis at pages 35-36. According to the Example, there is a single species disclosed, *i.e.*, SEQ ID NO: 1. *See* Written Description Synopsis at page 35.

It is concluded that the written description requirement is satisfied for the hypothetical claim of Example 9. *See* Written Description Synopsis at page 37. The Example notes that "hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing." Written Description Synopsis at page 36. Similarly, Appellants' claims recite "a DNA molecule which is at least 90% homologous [to SEQ ID NO:1]." Techniques for identifying DNA molecules that are at least 90% homologous to a reference nucleic acid sequence were conventional in the art at the time of the effective filing date of the present application. *See, e.g.*, specification at page 19, lines 3-15. (The ability of persons of ordinary skill in the art to identify DNA molecules that are at least 90% homologous to SEQ ID NO:1, using conventional methods, is discussed in section VIII.C.3(a), below.)

The analysis provided in Example 9 of the Written Description Synopsis further states that:

> a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Written Description Synopsis at pages 36-37.

Analogously, a person of ordinary skill in the art would not expect substantial variation among species of DNA molecules encompassed by or included within the subject matter of the claims on appeal because 90% homology to SEQ ID NO:1 would yield "structurally similar DNAs." Based on the reasoning set forth in Example 9 of the Written Description Synopsis, it must be concluded that Appellants were in possession of the claimed invention.

(c) Summary

The specification describes, in detail, the isolation of the cDNA molecule encoding AD7c-NTP (SEQ ID NO:1) and the complete nucleotide sequence of this nucleic acid molecule. Methods for obtaining DNA molecules that are at least 90% homologous to a reference nucleotide sequence were conventional in the art at the time of the effective filing date of the application and are described in the specification. The specification also clearly indicates how a person of ordinary skill in the art could ascertain whether a DNA molecule that is at least 90% homologous to SEQ ID NO:1

codes for a protein having an activity of AD7c-NTP when over-expressed in neuronal cells. In view of the details provided in the specification and the knowledge possessed by persons of ordinary skill in the art, a person of ordinary skill in the art would conclude that Appellants were in possession of the claimed subject matter.

In addition, the USPTO's Written Description Synopsis sets forth two examples that closely parallel the circumstances surrounding the claims on appeal. Both examples conclude that the written description requirement is fully satisfied. When the analytical guidance of the Written Description Synopsis is used to assess the claims on appeal, it must be concluded that the subject matter of the claims on appeal is more than adequately described in the specification. Thus, the written description requirement of 35 U.S.C. § 112, first paragraph, is fully satisfied.

4. Errors in the Written Description Rejection

A rejection for insufficient written description cannot be maintained unless the Examiner has presented sufficient evidence or reasoning indicating that a person of ordinary skill in the art would not recognize a description of the claimed invention in the specification. *See Wertheim*, 541 F.2d at 263, 191 USPQ at 97. Here, the Examiner has not presented sufficient evidence or reasoning to indicate that a person of ordinary skill in the art would not recognize in the specification a description of DNA molecules that are at least 90% homology to SEQ ID NO:1 and that code for proteins having AD7c-NTP activity when overexpressed in neuronal cells. Thus, the Examiner has not established a *prima facie* case of insufficient written description.

To support the written description rejection, the Examiner stated that:

> The art of record teaches that there is variation within the genus of the claimed DNA molecules. The art of record further teaches that one nucleotide change in a DNA molecule could result in the loss of its biological activity. The essential nucleotides required for an activity of AD7c-NTP are absent from the specification.

Paper No. 32, pages 3-4. This statement does not support the rejection. First, a rejection for insufficient written description cannot be based on the fact that "there is variation within the genus." Since members of a genus, by definition, are not identical, it follows that there is variation within all claimed genuses. The law is clear that the written description requirement can be satisfied for a claimed genus. *See, e.g.*, *Enzo*, 296 F.3d at 1324, 63 USPQ2d at 1613. Variation within a genus therefore cannot be a valid basis for a rejection under 35 U.S.C. § 112, first paragraph.

Second, the Examiner's assertion that "one nucleotide change in a DNA molecule could result in the loss of its biological activity" does not support a rejection for insufficient written description. The claims specify that the DNA molecule of the invention codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells. Thus, DNA molecules that have "lost their biological activity" are outside the scope of the claims on appeal. Since, a specification need only describe the subject matter encompassed by the claims, it is irrelevant that changes in DNA molecules may cause a loss of biological activity.

Finally, in the context of claims to genetic material, satisfaction of the written description requirement does require the identification of "essential nucleotides." In fact, the Federal Circuit has made it clear that the written description for genetic material may be satisfied by functional descriptions alone. *See Enzo*, 296 F.3d at 1324, 63 USPQ2d at

1613. Thus, satisfaction of the written description requirement does not require disclosure of the "essential nucleotides required for an activity of AD7c-NTP."

The claims on appeal include, not just a functional definition of the invention, but a structural definition as well. In terms of function, independent claim 1 specifies that the DNA molecule of the invention codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells. In terms of structure, the claim specifies that (a) the DNA molecule has SEQ ID NO:1 or is at least 90% homologous thereto, and (b) the DNA molecule is under control of a heterologous neuro-specific promoter. A person of ordinary skill in the art would have been able to easily determine whether a DNA molecule that is under the control of a heterologous neuro-specific promoter and that is at least 90% homologous to SEQ ID NO:1 codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells. *See* section VIII.C.3(b), below. Therefore, a person of ordinary skill in the art would have recognized from the specification that Appellants were in possession of the claimed invention.

The Examiner has not presented specific evidence or reasoning that supports the assertion that a person of ordinary skill in the art wold not have recognized that Appellants were in possession of the claimed invention. Thus, a *prima facie* case of insufficient written description has not been established. Appellants respectfully request that the Board reverse the Examiner's § 112, first paragraph rejection for alleged insufficient written description and remand this application for issue.

C. Enablement

1. The Enablement Rejection

Claims 1, 2, 3, 5, 6, 10-13, 35 and 44-47 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. *See* Paper No. 32, page 6.

There are two general bases for the enablement rejection. First, although the Examiner acknowledged that the specification enables a DNA molecule having SEQ ID NO:1 and a DNA molecule that encodes the amino acid sequence of SEQ ID NO:2, the Examiner stated that the specification:

> does not reasonably provide enablement for a DNA molecule which is at least 90% homologous to SEQ ID NO: 1 wherein said DNA molecule is under control of a heterologous neuro-specific promoter, and wherein said DNA molecule codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells.

See Paper No. 32, page 6.

The second basis relates to the ability of a person of ordinary skill in the art to practice the claimed methods. In particular, the Examiner stated that "the specification does not teach how to distinguish true negatives from false negative or true positives from false positives using the method contemplated in the claimed methods." *See* Paper No. 32, page 9. This assertion relates to the fact that the DNA molecules used in the claimed methods are under the control of a heterologous neuro-specific promoter. In addition, the Examiner asserted that "the specification does not teach how to distinguish

an increase in degradation of the protein coded for by the DNA construct from a decrease [in] expression of the protein coded for by the DNA construct." *See* Paper No. 32, page 9.

As discussed below, a person of ordinary skill in the art would have been able to make and use the DNA molecules of the invention without undue experimentation. A person of ordinary skill in the art would have also been able to practice the claimed methods without undue experimentation. Moreover, the Examiner has not established a *prima facie* case of non-enablement. Therefore, the enablement rejection was improper and should not be sustained.

2. Legal Standard for Enablement

In order to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, Appellants' specification must enable any person skilled in the art to make and use the claimed invention without undue experimentation. *See In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). *See also United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The factors to be considered when determining whether the necessary experimentation is "undue" include: (a) the breadth of the claims, (b) the nature of the invention, (c) the state of the prior art, (d) the level of one of ordinary skill, (e) the level of predictability in the art, (f) the amount of direction provided by the inventor, (g) the existence of working examples, and (h) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *See Wands*, 858 F.2d at 737, 8 USPQ2d at 1404. Moreover, as long as the specification discloses at least one method for making and

using the claimed invention, then the enablement requirement of 35 U.S.C. § 112, first paragraph is satisfied. *See Johns Hopkins Univ. v. CellPro, Inc.*, 152 F.3d 1342, 1361, 47 USPQ2d 1705, 1719 (Fed. Cir. 1998).

An Applicant is not limited to the confines of the specification to provide the necessary information to enable an invention. *See In re Howarth*, 654 F.2d 103, 105-6, 210 USPQ 689, 692 (CCPA 1981). An Applicant need not supply information that is well known in the art. *See Genentech, Inc. v. Novo Nordisk*, 108 F.3d 1361, 1366, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997); *Howarth*, 654 F.2d at 105-6, 210 USPQ at 692; *see also In re Brebner*, 455 F.2d 1402, 173 USPQ 169 (CCPA 1972) (finding a disclosure enabling because the procedure for making the starting material, although not disclosed, would have been known to one of ordinary skill in the art as evidenced by a Canadian patent). "'That which is common and well known is as if it were written out in the patent and delineated in the drawings.'" *Howarth*, 654 F.2d at 106, 210 USPQ at 692 (quoting *Webster Loom Co. v. Higgins* et al., 105 U.S. (15 Otto.) 580, 586 (1881)). Moreover, one of ordinary skill in the art is deemed to know not only what is considered well known in the art but also where to search for any needed starting materials. *See Id.*

In order to establish a *prima facie* case of lack of enablement, the Examiner has the initial burden to set forth a reasonable basis to question the enablement provided for the claimed invention. *See In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). To satisfy this burden, "it is incumbent upon the Patent Office. . . to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." *See In re Marzocchi*, 439 F.2d 220, 224, 169

USPQ 367, 370 (CCPA 1971) (emphasis in original). As enunciated by the Federal Circuit:

> [A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented *must* be taken as in compliance with the enabling requirement of the first paragraph of § 112 *unless* there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

In re Brana, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (emphasis in original; quoting *Marzocchi*, 439 F.2d at 224, 169 USPQ at 370).

3. A Person of Ordinary Skill in the Art Would Have Been Able to Make and Use DNA Molecules Which are at Least 90% Homologous to SEQ ID NO:1 and that Code For Proteins Having an Activity of AD7c-NTP When Overexpressed in Neuronal Cells Without Undue Experimentation

It would not have required undue experimentation on the part of a person of ordinary skill in the art to make and use the DNA molecules that are encompassed by or included within the subject matter of the claims on appeal. For example, a person of ordinary skill in the art could have obtained DNA molecules that are at least 90% homologous to SEQ ID NO:1 using only routine methods. Such DNA molecules could have easily been placed under the control of a heterologous neuro-specific promoter using standard DNA manipulation techniques. Finally, DNA molecules that are at least 90% homologous to SEQ ID NO:1 and that are under the control of a heterologous neuro-specific promoter could have easily been screened for the ability to code for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells. Thus,

it would have required only the application of routine techniques for a skilled artisan to obtain the DNA molecules that are encompassed by or included within the subject matter of the claims on appeal.

(a) A Person of Ordinary Skill in the Art Would Have Been Able to Obtain DNA Molecules Which are at Least 90% Homologous to SEQ ID NO:1 Without Undue Experimentation

(i) Isolation of DNA Molecules from a Library

The specification provides exemplary methods for obtaining DNA molecules which are at least 90% homologous to SEQ ID NO:1. Such methods involve the isolation of DNA molecules from cDNA libraries by stringent hybridization techniques. *See* specification at page 19, lines 3-15. As noted in the specification:

> DNA molecules which are at least 40%, 85% or 90% homologous to Seq. ID No. 1 may be isolated from cDNA libraries of humans and animals by hybridization under stringent conditions to the DNA molecule of Seq. ID No. 1 according to methods known to those of skill in the art. Stringent hybridization conditions are employed which select for DNA molecules having at least 40%, 85% and 90% homology to Seq. ID No. 1 are described in Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); and Maniatis *et al.*, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1985. The hybridizations may be carried out in 6 x SSC/5 x Denhardt's solution/0.1% SDS at 65°C. The degree of stringency is determined in the washing step. Thus, suitable conditions include 0.2 x SSC/0.01% SDS/65°C and 0.1 x SSC/0.01% SDS/65°C.

See specification at page 19, lines 3-15.

(ii) Random Mutagenesis

In addition to the methods presented in the specification, other methods for obtaining DNA molecules that are at least 90% homologous to SEQ ID NO:1 would have been available to a person of ordinary skill in the art. A specification need not supply information that is well known in the art in order to satisfy the enablement requirement. *See Genentech, Inc. v. Novo Nordisk*, 108 F.3d 1361, 1366, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997); *See also Hybritech v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986) ("a patent need not teach, and preferably omits, what is well known in the art.") Thus, methods that were well known in the art at the time of the effective filing date of the application would have been available to persons of ordinary skill in the art in order to obtain DNA molecules that are at least 90% homologous to SEQ ID NO:1.

Among such well known methods are directed and random mutagenesis techniques. Exemplary methods are described in Sambrook *et al.*, "Creating Many Mutations in a Defined Segment of DNA," in *Molecular Cloning, A Laboratory Manual*, Sambrook *et al.*, eds., Cold Spring Harbor Laboratory Press, pp. 15.95-15.108 (1989) (copy attached hereto as Exhibit 3). Sambrook provides a solution to the problem in the art of predicting the effects of amino acid substitutions. Sambrook notes that using individual oligonucleotides to replace more than 20 amino acids with several other amino acids is impractical. To overcome these problems associated with making individual mutations one-by-one, Sambrook describes oligonucleotide-, chemical-, and polymerase-based mutagenesis methods. Such methods would have been available to

persons of ordinary skill in the art to obtain DNA molecules that are at least 90% homologous to SEQ ID NO:1.

(iii) Directed Mutagenesis

A person of ordinary skill in the art could have also obtained DNA molecules that are at least 90% homologous to SEQ ID NO:1 by directed mutation of individual nucleotides. For example, a person of ordinary skill in the art could have created one or more mutations in the DNA sequence of SEQ ID NO:1 that do not effect the amino acid sequence that is translated therefrom. Based on the degeneracy of the genetic code, a person of ordinary skill in the art would have appreciated that most amino acids are encoded by several codons. Replacing one codon that encodes a particular amino acid with another codon that also encodes that same amino acid would create a DNA molecule that is non-identical to SEQ ID NO:1, but is at least 90% homologous to SEQ ID NO:1. The mutated DNA molecule would encode the exact same amino acid sequence as SEQ ID NO:1 (*i.e.*, the AD7c-NTP amino acid sequence); thus, the mutated DNA molecule would necessarily code for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells.

In addition, a person of ordinary skill in the art could have created directed mutations in SEQ ID NO:1 that result in a substitution of one amino acid for another amino acid having similar biochemical properties. Suitable amino acid substitutions would have been known to persons of ordinary skill in the art. Again (depending on the number of mutations made), the DNA molecule would be at least 90% homologous to SEQ ID NO:1. Since the mutations would result in the substitution of one amino acid

with another amino acid having similar biochemical properties, it would be expected that the protein coded by such a mutated nucleic acid molecule would have an activity of AD7c-NTP when overexpressed in neuronal cells. As discussed immediately below, a person of ordinary skill in the art could have confirmed that the protein coded by a mutated version of SEQ ID NO:1 has an activity of AD7c-NTP when overexpressed in neuronal cells using routine methods.

(b) A Person of Ordinary Skill in the Art Would Have Been Able to Determine if a DNA Molecule That is at Least 90% Homologous to SEQ ID NO:1 Codes for a Protein Having AD7c-NTP Activity When Overexpressed in Neuronal Cells Without Undue Experimentation

Once obtained, DNA molecules that are at least 90% homologous to SEQ ID NO:1 could have easily been tested for the ability to encode a protein having an activity of AD7c-NTP. The specification describes various methods for assaying for AD7c-NTP activity. For example, transgenic animals could have been made that overexpress AD7c-NTP in neuronal cells. The transgenic animals could have been analyzed for evidence of neuronal or neuritic abnormalities associated with Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas and glioblastomas. *See* specification at page 20, lines 1-29.

Additionally, *in vitro* methods could have been used to test for AD7c-NTP activity. For example, the specification describes a working example involving the overexpression of AD7c-NTP in neuronal cells and the subsequent analysis for cellular characteristics of Alzheimer's disease, including apoptosis and neuritic sprouting. *See* specification at page 45, line 16, through page 46, line 26. Specifically, the AD7c-NTP

cDNA was inserted into an expression vector under the control of the CMV promoter. The resulting construct, designated pcDNA3-AD7c-NTP, was introduced into a neuronal cell line known as SH-Sy5y. *See* specification at page 45, lines 17-22. The transfected cells were then observed to determine the phenotypes associated with AD7c-NTP overexpression. According to the specification:

> SH-Sy5y cells transfected with pcDNA3-AD7c-NTP exhibited extensive neuritic growth with fine interconnecting processes detected on most cells (figs. 6B-6D). In addition, pcDNA3-AD7c-NTP transfected cultures always contained numerous round, refractile floating cells (dead) which failed to exclude Trypan blue dye. Immunocytochemical staining of stationary cultures using the N3I4 monoclonal antibody revealed intense labeling of the cell bodies and cell processes of SH-Sy5y cells transfected with pcDNA3-AD7c-NTP (Figs. 6F and 6G), and absent immunoreactivity in SH-Sy5y cells transfected with pcDNA3 (empty vector) (Fig. 6E). These studies demonstrate that over expression of AD7c-NTP in transfected neuronal cells promotes neuritic sprouting and cell death, two of the major features of Alzheimer's disease neurodegeneration.

See specification at page 46, lines 12-23.

The specification also describes the effects of AD7c-NTP overexpression on cell viability and the expression of genes associated with Alzheimer's disease (Tau, bA4 amyloid), neuritic sprouting (synaptophysin) and apoptosis (p53, SC95-Fas, NO-Tyr, NOS3) in neuronal cells. *See* specification at page 49, lines 16-26 and Figs. 7A-7C and 8A-8D.

In view of these examples, a person of ordinary skill in the art could have easily transfected a neuronal cell line with a DNA molecule having at least 90% homology to SEQ ID NO:1 under the control of a heterologous promoter, and could have screened for one or more of the phenotypes of the transfected cells. The occurrence of one or more of

the cellular/molecular phenotypes described and illustrated in the specification would indicate that the protein coded from the DNA molecule had an activity of AD7c-NTP when overexpressed in neuronal cells. Thus, a person of ordinary skill in the art could have easily determined whether a DNA molecule having at least 90% homology to SEQ ID NO:1 codes for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells.

Alternatively, a person of ordinary skill in the art could have tested a mutated version of SEQ ID NO:1 for the ability to produce one or more AD7c-NTP phenotypes when overexpressed in neuronal cells *before* determining the degree of homology shared between the mutated molecule and SEQ ID NO:1. For example, a neuronal cell line could have been transfected with a randomly mutagenized version of SEQ ID NO:1 under the control of a promoter. Next, the transfected cells could have been observed to determine if they exhibited one or more phenotypes associated with AD7c-NTP overexpression. If so, the nucleic acid molecules would have been isolated from the cells and the degree of homology to SEQ ID NO:1 could have been easily determined (*i.e.*, to determine if they had at least 90% homology to SEQ ID NO:1). This type of strategy would have also been known and available to persons of ordinary skill in the art at the time of the effective filing date of the application.

(c) Summary

A person of ordinary skill in the art could have obtained a DNA molecule that is at least 90% homologous to SEQ ID NO:1 and that codes for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells by (i) obtaining a DNA

molecule that is at least 90% homologous to SEQ ID NO:1, and (ii) determining whether the DNA molecule has an activity of AD7c-NTP when overexpressed in neuronal cells. As demonstrated above, both obtaining a DNA molecule that is at least 90% homologous to SEQ ID NO:1, and determining whether the DNA molecule has an activity of AD7c-NTP when overexpressed in neuronal cells, could have been accomplished with routine methods in the art, including methods that are explicitly described in the specification. Thus, obtaining DNA molecules encompassed by or included within the subject matter of the claims on appeal would not have required undue experimentation.

4. Errors in the Enablement Rejection Relating to the Ability of a Person of Ordinary Skill in the Art to Make and Use the DNA Molecules of the Invention

(a) Making and Using DNA Molecules That are at Least 90% Homologous to SEQ ID NO:1 Would Not Have Required Knowledge of "Essential Nucleotides"

In support of the enablement rejection, the Examiner stated that "[t]he specification does not disclose which nucleotides of the claimed DNA molecule is [sic] considered essential for one skilled in the art to make a representative number of DNA molecules with 90% homology to SEQ ID NO:1." *See* Paper No. 32, page 7.

The Examiner, however, has not explained why it is believed that the production of DNA molecules that are included within the transgenic animals of the invention would have required the identification of "essential" nucleotides. As discussed above, there are numerous methods described in the specification and available in the art at the time of the application that could have been used to produce DNA molecules that are 90%

homologous to SEQ ID NO:1. *See* section VIII.C.3(a), above. Such methods do not require knowledge of "essential" nucleotides. In addition, methods for determining if a DNA molecule has an activity of AD7c-NTP when it is overexpressed in neuronal cells would not have required knowledge of "essential" nucleotites. *See* section VIII.C.3(b), above. Thus, the Examiner's statement regarding the identification of "essential" nucleotides does not support a rejection for lack of enablement.

(b) Making and Using DNA Molecules That are at Least 90% Homologous to SEQ ID NO:1 Would Not Have Required the Ability to Predict the Effects of Mutations on Protein Function

The Examiner also asserted that:

> [s]ince, the relationship between a sequence of a peptide and its tertiary structure (i.e. its activity) are not well understood and are not predictable . . . it would [have] required undue experimentation for one skilled in the art to arrive at other DNA molecules with 90% homology to SEQ ID NO: 1 and having SEQ ID NO: 1 activity when over-expressed in neuronal cells.

See Paper No. 32, page 8.

The Examiner has not explained why it is believed that the production of the DNA molecules of the invention would have required a skilled artisan to predict the relationship between the sequence of a peptide and its structure. A skilled artisan would not have needed to predict the structural and/or functional consequences of particular mutations or base changes in order to produce DNA molecules that are at least 90% homologous to SEQ ID NO:1 and that code for proteins having an activity of AD7c-NTP when overexpressed in neuronal cells. To make DNA molecules that are encompassed by or included within the subject matter of the claims on appeal, a skilled artisan would

have only needed to: (a) obtain DNA molecules that are at least 90% homologous to SEQ ID NO:1, and (b) test them for the ability to encode proteins that possess AD7c-NTP activity when overexpressed in neuronal cells. As discussed in section VIII.C.3, above, both of these processes would have been routine in the art and neither requires the ability to predict the effects of specific mutations on protein function.

Thus, any uncertainty that is associated with predicting protein function from sequence data is irrelevant in an analysis of the enablement of the claims on appeal. The Examiner's statements relating to the ability of a skilled artisan to predict the relationship between the sequence of a peptide and its structure or activity do not support a rejection for lack of enablement.

(c) The Specification Describes Methods for Determining if a DNA Molecule That is at Least 90% Homologous to SEQ ID NO:1 has an Activity of AD7c-NTP when Overexpressed in Neuronal Cells

The Examiner also stated that "the specification does not provide sufficient guidance and/or factual evidence for one skilled in the art to determine without an undue amount of experimentation . . . if the nucleic acid sequence with at least 90 percent homology to SEQ ID NO: 1, would exhibit the same biological function of SEQ ID NO: 1 (observed activity when the sequence is over-expressed in neuronal cells)." *See* Paper No. 32, pages 7-8. This is an incorrect statement. As discussed in section VIII.C.3(b), above, methods for determining whether a given DNA molecule codes for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells are set forth in the specification and would not have required undue experimentation.

No evidence has been presented to indicate that it would have required undue experimentation to determine whether a given DNA molecule codes for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells. Thus, a *prima facie* case of non-enablement cannot be established on this basis.

(d) Summary

In order to establish a *prima facie* case of non-enablement, the Examiner must present sufficient evidence or scientifically sound reasoning which indicates that the claimed invention is not enabled. *See Marzocchi*, 439 F.2d at 224, 169 USPQ at 370. The Examiner has not presented any evidence or reasonable arguments that support the assertion that making and using DNA molecules that are at least 90% homologous to SEQ ID NO:1 and that code for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells would have required undue experimentation. Thus a *prima facie* case of non-enablement with respect to claims that encompass or include the use of DNA molecules that are at least 90% homologous to SEQ ID NO:1 and that code for a protein that has an activity of AD7c-NTP when overexpressed in neuronal cells has not been established.

5. A Person of Ordinary Skill in the Art Would Have Been Able to Practice the Claimed Methods Without Undue Experimentation

Claims 10-13 and 44-47 are directed to screening methods comprising: (a) contacting a candidate drug with the host cell of claim 5 or 42, and (b) detecting at least one of the following: (i) the suppression or prevention of expression of the protein coded for by the DNA construct of said host cell; (ii) the increased degradation of the protein

coded for by the DNA construct of said host cell; or (iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in said host cell, wherein said host cell is a neuronal cell; due to the drug candidate compared to a control cell line which has not contacted the candidate drug. The practice of the claimed methods would not have required undue experimentation.

(a) Contacting a Candidate Drug with a Host Cell

First, a person of ordinary skill in the art could have easily contacted a candidate drug with a host cell. The specification describes exemplary conditions under which a candidate drug may be contacted with a host cell. *See* specification at page 22, line 17, through page 23, line 8.

(b) Detecting the Suppression or Prevention of Expression of the Protein Coded for by the DNA Construct of the Host Cell

Second, a person of ordinary skill in the art could have easily detected the suppression or prevention of expression of the protein coded for by the DNA molecule of the host cell (*i.e.*, a DNA molecule of SEQ ID NO:1 or a DNA molecule which is at least 90% homologous thereto, wherein the DNA molecule is under the control of a heterologous neuro-specific promoter, and wherein the DNA molecule codes for a protein that has an activity of AD7c-NTP when over expressed in neuronal cells). As noted in the specification:

> In order to test for the level of NTP expression, immunohistochemical staining may be carried out as

> described in the Examples. Alternatively, the plates containing the cells may be centrifuged to pellet cellular debris from the medium, and a sample of the media [can be] tested for the NTP concentration. the concentration of NTP may be determined by ELISA with an antibody which is specific for NTP. Methods for carrying out such assays are disclosed in WO94/10569 and are well known to those of ordinary skill in the art. The concentration of NTP in the test cells/media is then compared to the concentration of control cells that have been treated the same way except that the media does not contain the candidate drug (but may contain the same level of DMSO). The result of the ELISA are fit to a standard curve and expressed as ng/mL NTP. See WO96/40895.

See specification at page 23, lines 13-23.

Immunohistochemical methods that could have been used in the context of the claimed methods are described in the examples. For instance, Example 8 describes and illustrates the immunohistochemical staining of neuronal cells that overexpress AD7c-NTP using an AD7c-NTP monoclonal antibody (N3I4). *See* specification at page 46, lines 17-21 and Figs. 6F and 6G.

In order to confirm that a decrease in protein level represents a decrease in gene expression (as opposed to an increase in protein degradation, for example), a person of ordinary skill in the art could have assayed the level of AD7c-NTP mRNA in the cells that had been contacted with the candidate drug. A decrease in mRNA levels would confirm that the decreased protein level is caused by the suppression or prevention of expression of the protein coded for by the DNA construct. Methods for assaying AD7c-NTP mRNA levels by Northern blot are exemplified in the specification at page 41, lines 1-28 (Example 5).

Thus, a person of ordinary skill in the art could have detected the suppression or prevention of expression of the protein coded for by the DNA molecule of the host cell

of the invention using the methods set forth in the specification and the knowledge generally available in the art. Such methods would not have required undue experimentation.

(c) Detecting the Increased Degradation of the Protein Coded for by the DNA Construct of the Host Cell

Third, a person of ordinary skill in the art could have easily detected the increased degradation of the protein coded for by the DNA construct of the host cell. As discussed above, immunohistochemical methods are described in the specification that would have enabled a person of ordinary skill in the art to determine if a candidate drug caused a decrease in the amount of protein coded for by the DNA construct of the host cell. In addition, the specification illustrates the well-known technique of Northern analysis which could have been used in the context of the claimed methods to ascertain if the decrease in protein level corresponded to a decrease in mRNA level. A decrease in protein levels without a decrease in mRNA levels would indicate that the drug increased the degradation of the protein coded for by the DNA construct.

Thus, a person of ordinary skill in the art could have detected the increased degradation of the protein coded for by the DNA construct of the host cell of the invention using the methods set forth in the specification and the knowledge generally available in the art. Such methods would not have required undue experimentation.

(d) Detecting the Reduction of Frequency of Neuritic Sprouting, Nerve Cell Death, Degenerating Neurons, Neurofibrillary Tangles, or Irregular Swollen Neurites and Axons in the Host Cell

Fourth, a person of ordinary skill in the art could have easily detected the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host cell. As noted in the specification:

> After the treatment period [during which host cells are contacted with a candidate drug], the cells are tested either for the level of NTP expression and/or, if the cells are neuronal cells, examined for the presence and/or frequency of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons.

See specification at page 23, lines 9-12. Phenotypes associated with AD7c-NTP overexpression in neuronal cells are illustrated in the specification at page 45, line 16, through page 46, line 26 (Example 8, Figs. 6B-6D), and at page 48, line 16, through page 51, line 15 (Example 10, Figs. 7A-7C and 8A-8D). Methods for detecting phenotypes associated with AD7c-NTP overexpression, as illustrated in the specification, are discussed in section VIII.C.3(b), above.

Thus, a person of ordinary skill in the art could have detected the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host cell of the invention using the methods set forth in the specification and the knowledge generally available in the art. Such methods would not have required undue experimentation.

(e) Summary

As demonstrated above, it would not have required undue experimentation for a person of ordinary skill in the art to:

- contact a candidate drug with a host cell of the invention,
- detect the suppression or prevention of expression of the protein coded for by the DNA construct of the host cell,
- detect the increased degradation of the protein coded for by the DNA construct of the host cell, or
- detect the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in the host cell.

Accordingly, a person of ordinary skill in the art would have been able to practice the claimed methods without undue experimentation.

6. Errors in the Enablement Rejection Relating to the Ability of a Person of Ordinary Skill in the Art to Practice the Methods of the Invention

(a) A Drug that Suppresses or Prevents the Expression of the Protein Coded for by the Host Cell is a "Candidate Drug that is Potentially Useful for the Treatment of Alzheimer's Disease"

The claimed methods include detecting the suppression or prevention of expression of the protein coded for by the DNA construct of the host cell. *See* section VIII.C.5(b), above. The claims specify that the DNA molecule expressed from the host cell is under the control of a heterologous neuro-specific promoter. According to the

Examiner, "[t]he suppression or prevention of expression of the protein coded by the DNA construct in b(i) would reflect interaction with the control sequence [i.e., heterologous neuro-specific promoter] and result in false positives/false negatives." *See* Paper No. 32, page 9. This is an incorrect assertion.

The Examiner has not clearly explained what is meant by "false positives" and "false negatives." It appears, however, that the term "false positives" was intended to mean drug candidates that suppress gene expression by interacting with the heterologous neuro-specific promoter but would not suppress the expression of AD7c-NTP in its normal cellular environment. Similarly, it appears that the term "false negatives" was intended to mean drug candidates that do not suppress expression from the heterologous neuro-specific promoter but would nonetheless suppress the expression of AD7c-NTP in its normal cellular environment.

The error in the Examiner's reasoning is that the claims are directed to methods for screening a candidate drug that is *potentially* useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas or glioblastomas. The claims do not require that the drug identified by the claimed methods necessarily be effective for the treatment of Alzheimer's disease or other conditions. A drug that suppresses or prevents the expression of the protein coded for by the DNA construct of the host cell, even if it does so by interacting with the heterologous neuro-specific promoter, would be a "candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas or glioblastomas."

In response to Appellants' argument on this issue, the Examiner stated that:

> the assertion that, "the initial screen for drugs that are potentially useful" indicates that one skilled in the art would not know any more about the drug than what was known before testing the drug in the claimed method.

See Advisory Action dated January 16, 2004. The Examiner's statements appear to be more of an argument for lack of utility than for lack of enablement since the claims clearly indicate that the methods are for screening candidate drugs that are *potentially* useful for the treatment or prevention of Alzheimer's disease and other conditions. *See* also specification at page 21, lines 3-6.

Nonetheless, Appellants respectfully submit that the assertion that "one skilled in the art would not know any more about the drug than what was known before testing the drug in the claimed method" is an incorrect statement. Upon identifying a drug by the claimed methods, it would be known that the drug (i) suppresses or prevents the expression of the protein coded for by the DNA construct of the host cell (i.e., a protein that has an activity of AD7c-NTP), (ii) increases the degradation of the protein coded for by the DNA construct of the host cell, and/or (iii) causes the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or iregular swollen neurites and axons in the host cell. This is more information than was known about the drug before practicing the claimed methods. Such information obtained from the practice of the claimed methods would identify the drug as one that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas or glioblastomas.

In summary, the claims are directed to methods for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas or glioblastomas. A drug that

suppresses or prevents the expression of the protein coded for by the DNA construct of the host cell, even if it does so by interacting with the heterologous neuro-specific promoter, would be a "candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas or glioblastomas." Thus, the Examiner's statements regarding "false positives" and "false negatives" does not support the enablement rejection.

(b) A Drug that Suppresses or Prevents the Expression of the Protein Coded for by the Host Cell May do so by Mechanisms that do not Involve Interaction with the Heterologous Promoter

The Examiner's assertion that the claimed methods would identify "false positives" and "false negatives" is further flawed because it assumes that a drug that suppresses or prevents protein expression would do so only by interacting with the heterologous neuro-specific promoter. This is an incorrect assumption.

A candidate drug could suppress or prevent the expression of the protein coded for by the DNA construct through mechanisms other than interaction with the promoter. For instance, a drug might stimulate degradation of the mRNA, reduce the stability of the mRNA, or interfere with translation of the mRNA. Drugs that exert such effects would be identified by the claimed methods and would be regarded as candidate drugs that are potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas or glioblastomas.

(c) Methods for Distinguishing the Suppression of Protein Expression from an Increase in Protein Degradation were Well Known and Could Have been Used in the Context of the Claimed Methods

In addition to detecting the suppression or prevention of expression of the protein coded for by the DNA construct of the host cell, the claimed methods also include detecting the increased degradation of the protein coded for by the DNA construct of the host cell. According to the Examiner, "the specification does not teach how to distinguish an increase in degradation of the protein coded for by the DNA construct from a decrease [in] expression of the protein coded for by the DNA construct." *See* Paper No. 32, page 9. Although the specification does not explicitly describe how one of ordinary skill in the art would go about distinguishing an increase in protein degradation from a decrease or suppression of gene expression, such methods are basic techniques in the field of molecular biology and would have been known and available to persons of ordinary skill in the art at the time of the effective filing date of the application. It is well established that a specification need not teach, and preferably omits, information that is well known in the art. *See Genentech, Inc. v. Novo Nordisk*, 108 F.3d 1361, 1366, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997); *See also Hybritech v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986). Thus, the Examiner's statements do not support a rejection for lack of enablement.

As noted in sections VIII.C.5(b) and (c), above, detecting both (i) the suppression or prevention of expression of the protein coded for by the DNA construct of the host cell, and (ii) the increased degradation of the protein coded for by the DNA construct of the host cell could have been accomplished using immunohistotochemical techniques. A decrease in protein levels, as determined by immunohistochemical methods, would

reflect either a suppression of protein expression or an increased degradation of the protein, as compared to a control cell that was not contacted with the candidate drug.

To distinguish (i) from (ii), a person of ordinary skill in the art would have simply measured mRNA levels. For example, a person of ordinary skill in the art would have been able to measure AD7c-NTP mRNA levels using a Northern blot, as illustrated in the specification at page 41, lines 1-28 (Example 5). A decrease in protein levels without a decrease in mRNA levels would indicate that the drug increased the degradation of the protein coded for by the DNA construct. A decrease in protein levels coupled with a decrease in mRNA levels would indicate the suppression of expression of the protein coded for by the DNA construct.

Methods for distinguishing the suppression or prevention of expression of a protein from an increase in protein degradation were well known in the art at the time of the effective filing date of the application. A review of the scientific literature reveals that techniques for distinguishing protein degradation from transcriptional-level regulation were well known in the art. For instance, in Hwong *et al.*, *J. Biol. Chem.* *268*:18982-18986 (1993) (copy submitted as Exhibit 1 with Reply to Final Office Action filed December 15, 2003), it is shown that the amount of topoisomerase I protein in phytohemagglutinin (PHA)-stimulated human T lymphocytes was regulated by protein degradation. This conclusion was made by comparing topoisomerase I mRNA levels (determined by measuring incorporation of radiolabeled thymidine and by Northern analysis (*see* Fig. 3)) to topoismerase I protein levels (determined by Western blot *see* Fig. 4.). Although both mRNA and protein levels increased following PHA stimulation, the degree of increase in protein levels was *less than* the degree of increase in mRNA

levels. *See* Hwong *et al.*, paragraph bridging pages 18984-18985. By measuring [^{35}S] methionine incorporation in the presence of PHA (*see* Fig. 5.), it was confirmed that the discrepancy between mRNA and protein levels was due to degradation of topoisomerase I protein. *See* Hwong *et al.*, page 18985, paragraph bridging left and right columns.

Another example is found in Eguchi *et al.*, *Cancer Res.*, *63*:4739-4746 (2003) (copy submitted as Exhibit 2 with Reply to Final Office Action filed December 15, 2003). In Eguchi *et al.*, it was found that addition of *Helicobacter pylori* to AGS human grastric epithelial cells caused a reduction in p27 protein (as determined by Western blot). *See* Eguchi *et al.*, paragraph bridging pages 4740 and 4741. The levels of p27 mRNA (as determined by Northern blot), however, were not altered by *H. pylori* treatment. *See* Eguchi *et al.*, page 4741, left column. By analyzing metabolically radiolabeled p27, it was confirmed that "the down-regulation of p27 by *H. pylori* is due to increased p27 protein degradation." *See id.*

Although Eguchi *et al.* was published in 2003, the general methods used in this reference (*i.e.*, Northern blot, Western blot, and pulse-labeling of proteins) are the same as those used by Hwong *et al.* (published in 1993). Thus, the methodology used by Eguchi *et al.*, would have been known and available to persons of ordinary skill in the art well before the effective filing date of the present application. The techniques used by Hwong *et al.* and Eguchi *et al.* could have been applied by persons of ordinary skill in the art in the context of the present claims to determine if the effect of a candidate drug was due to (i) the suppression or prevention of expression of the protein coded for by the DNA construct, or (ii) an increase in degradation of the protein. Therefore,

destinguishing (i) from (ii) would not have involved anything more than the application of routine techniques.

The Examiner asserted that, even though the specification clearly illustrates the measurement of AD7c-NTP mRNA levels by Northern analysis, the specification does not specifically describe the use of Northern analyses to distinguish the suppression or prevention of expression of a protein from an increase in protein degradation. *See* Paper No. 32, page 14. Appellants submit that, regardless of the context in which the working examples of the present specification were performed, the Examiner's observation does not support a *prima facie* case of non-enablement.

Even though the working example of Northern analysis in the specification was conducted in a context different from that of the claimed methods, the example would nonetheless have provided additional guidance for a person of ordinary skill to carry out the claimed methods. In addition, the Examiner has the initial burden of showing that the practice of the claimed methods would have required undue experimentation. Simply pointing out that the working examples were performed in a context different from that of the claimed methods does not satisfy the Examiner's burden of showing that the methods are not enabled.

In response to Appellants' submission of Hwong and Eguchi as demonstrating that distinguishing the suppression or prevention of expression of a protein from an increase in protein degradation was routine in the art, the Examiner stated that:

> [T]he argument that techniques for distinguishing protein degradation from decrease in protein expression are well known in the art (See Exhibits 1 and 2), is not found persuasive because the examiner already acknowledged that that [sic] at the time the application was filed, the techniques were well known. However, at the time the

> application was filed it, it [sic] was not well known in the art how to use the same method to observe protein degradation and a decrease in protein expression. See applicants' exhibits that teach using two different and distinct methods to determine protein degradation and a decrease in protein expression.

See Advisory Action dated January 16, 2004. Appellants note that the claims do not specify that detecting either (i) the suppression or prevention of expression of the protein coded for by the DNA construct of the host cell, or (ii) the increased degradation of the protein coded for by the DNA construct of the host cell, must be accomplished using only one analytical process step. In fact, the claims do not in any way limit the manner by which (i) and (ii) can be detected. The methods of Hwong and Eguchi were known and available to persons of ordinary skill in the art at the time of the effective filing date of the application and therefore could have been used in the practice of the claimed methods. That the methods of Hwong and Eguchi involve more than one analytical step does not suggest that these methods would not have been used in the practice of the claimed methods.

Finally, with respect to Hwong and Eguchi, the Examiner stated that: "the exhibits do not teach that it is well known in the art to use a nucleotide sequence encoding a AD7c-NTP protein under control of a heterologous promoter in a drug screening assay." *See* Advisory Action dated January 16, 2004. Hwong and Eguchi, however, were not presented to teach that it was "well known in the art to use a nucleotide sequence encoding a AD7c-NTP protein under control of a heterologous promoter in a drug screening assay." As noted above, Hwong and Eguchi were presented to show that, as of the effective filing date of the application, methods for distinguishing the suppression or prevention of expression of a protein from an increase

in protein degradation were well known in the art and could have been used in the practice of the claimed methods. The Examiner has not presented any evidence or arguments to indicate that this is not the case.

(d) Summary

In order to establish a *prima facie* case of non-enablement, the Examiner must present sufficient evidence or scientifically sound reasoning which indicates that the claimed invention is not enabled. *See Marzocchi*, 439 F.2d at 224, 169 USPQ at 370. The Examiner has made two general assertions to support the enablement rejection of the method claims. First, the Examiner asserted that drugs that effect AD7c-NTP expression by interacting with the heterologous neuro-specific promoter would be "false positives" (or "false negatives"). As explained above, drugs that suppress or prevent the expression of the protein coded for by the DNA construct of the host cells of the invention, even if they do so by interacting with the heterologous neuro-specific promoter, would be candidate drugs that are potentially useful for the treatment or prevention of Alzheimer's disease. The Examiner has not presented any evidence or arguments to refute this contention. Second, the Examiner asserted that the specification does not teach how to distinguish (i) the suppression or prevention of expression of a protein from (ii) an increase in protein degradation. As explained above, methods for distinguishing (i) from (ii) were routine in the art, and persons of ordinary skill in the art would have appreciated that such methods could have been used in the practice of the claimed methods. The Examiner has not presented any evidence or arguments to refute this contention. Thus, a *prima facie* case of non-enablement has not been established.

IX. Conclusion

In view of the foregoing discussion, Appellants respectfully request that the Board reverse the Examiner's 35 U.S.C. § 112, first paragraph rejections of claims 1-3, 5, 6, 10-13, 35 and 44-47 and remand this application for issue.

Respectfully submitted,

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X. Appendix (37 C.F.R. § 1.192(c)(9))

A. Claims on Appeal

1. A DNA construct, which comprises the DNA molecule of SEQ ID NO:1 or a DNA molecule which is at least 90% homologous thereto, wherein said DNA molecule is under control of a heterologous neuro-specific promoter, and wherein said DNA molecule codes for a protein that has an activity of AD7c-NTP when over-expressed in neuronal cells.

2. The DNA construct of claim 1, which is contained within a vector.

3. The DNA construct of claim 1, which is contained by a virion.

5. A host cell transformed with the DNA construct of claim 1.

6. The host cell of claim 5, which is a neuronal cell.

10. An *in vitro* method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, or glioblastomas, said method comprising:

(a) contacting a candidate drug with the host cell of claim 5, and

(b) detecting at least one of the following:

(i) the suppression or prevention of expression of the protein coded for by the DNA construct of said host cell;

(ii) the increased degradation of the protein coded for by the DNA construct of said host cell; or

(iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in said host cell, wherein said host cell is a neuronal cell;

due to the drug candidate compared to a control cell line which has not contacted the candidate drug.

11. The method of claim 10, wherein said protein has SEQ ID NO:2.

12. The method of claim 10, wherein said protein is over-expressed by said host cell.

13. The method of claim 10, wherein said cell is a neuronal cell.

35. The DNA construct of claim 1, wherein said activity of AD7c-NTP is selected from the group consisting of neuritic sprouting, nerve cell death, nerve cell degeneration, neurofibrillary tangles, and irregular swollen neurites.

44. An *in vitro* method for screening a candidate drug that is potentially useful for the treatment or prevention of Alzheimer's disease, neuroectodermal tumors, malignant astrocytomas, or glioblastomas, said method comprising:

(a) contacting a candidate drug with the host cell of claim 42, and

(b) detecting at least one of the following:

(i) the suppression or prevention of expression of the protein coded for by the DNA construct of said host cell;

(ii) the increased degradation of the protein coded for by the DNA construct of said host cell; or

(iii) the reduction of frequency of at least one of neuritic sprouting, nerve cell death, degenerating neurons, neurofibrillary tangles, or irregular swollen neurites and axons in said host cell, wherein said host cell is a neuronal cell;

due to the drug candidate compared to a control cell line which has not contacted the candidate drug.

45. The method of claim 44, wherein said DNA molecule comprises a DNA sequence having the nucleotide sequence set forth in SEQ ID NO:1.

46. The method of claim 44, wherein said protein is over-expressed by said host cell.

47. The method of claim 44, wherein said cell is a neuronal cell.

B. Objected Claims

36. The DNA construct of claim 1, wherein said DNA molecule codes for a protein having the amino acid sequence of SEQ ID NO:2.

37. The DNA construct of claim 1, wherein said DNA molecule consists of the DNA molecule of SEQ ID NO:1.

C. Allowed Claims

39. A DNA construct, which comprises a DNA molecule that encodes the amino acid sequence set forth in SEQ ID NO:2, wherein said DNA molecule is under control of a heterologous neuro-specific promoter, and wherein said DNA molecule codes for a protein that has an activity of AD7c-NTP when expressed in neuronal cells.

40. The DNA construct of claim 39, which is contained within a vector.

41. The DNA construct of claim 39, which is contained within a virion.

42. A host cell transformed with the DNA construct of claim 39.

43. The host cell of claim 42, which is a neuronal cell.

49. The DNA construct of claim 39, wherein said DNA molecule comprises a DNA sequence having the nucleotide sequence set forth in SEQ ID NO:1.

Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A $\rightarrow$ B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A $\rightarrow$ B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

e.g. expression vectors, the necessary common attribute is the ORF (SEQ ID NO: 2).

Weighing all factors including (1) that the full length ORF (SEQ ID NO: 2) is disclosed and (2) that any substantial variability within the genus arises due to addition of elements that are not part of the inventor's particular contribution, taken in view of the level of knowledge and skill in the art, one skilled in the art would recognize from the disclosure that the applicant was in possession of the genus of DNAs that comprise SEQ ID NO: 2.

Conclusion: The written description requirement is satisfied.

Example 9: <u>Hybridization</u>

Specification: The specification discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification includes an example wherein the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) for the isolation of nucleic acids that encode proteins that bind to dopamine receptor and stimulate adenylate cyclase activity. The hybridizing nucleic acids were not sequenced. They were expressed and several were shown to encode proteins that bind to a dopamine receptor and stimulate adenylate cyclase activity. These sequences may or may not be the same as SEQ ID NO: 1.

Claim:

An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1,

wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Analysis:

A review of the full content of the specification indicates that the essential feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing.

The claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with a specific activity.

The search of the prior art indicates that SEQ ID NO: 1 is novel and unobvious.

There is a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus.

There is actual reduction to practice of the disclosed species.

Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of

skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Conclusion: The claimed invention is adequately described.

At present, it is impossible to predict with accuracy the effect of substituting one amino acid for another in a protein. Current attempts to "improve" the properties of a protein therefore depend on analyzing large numbers of variants that are created by site-directed mutagenesis in promising regions (e.g., in and around the active site of an enzyme). Clearly, the number of potential variations that can be created, even in a circumscribed region of a protein, is extremely large. For example, 114 different mutants would be required simply to insert every possible amino acid at each of six locations in a protein. This number grows to 6^{19} if such substitutions are made in a combinatorial fashion. When planning this type of mutagenesis, careful choices must therefore be made to keep the numbers of mutants within manageable limits. For example, the numbers of potential mutants can be markedly reduced by avoiding replacements that are (1) highly conservative (i.e., the substitution of one amino acid with another whose chemical properties are very similar), (2) highly radical (i.e., replacing an amino acid with another whose chemical properties are completely different), or (3) misguided (e.g., the substitution of cysteine residues in secretory proteins). However, when the number of desired mutants exceeds 20 or so, it becomes impractical and expensive to generate each of them individually using a separate mutagenic oligonucleotide. Methods have therefore been devised to use degenerate pools of oligonucleotides to create large populations of mutants in a single round of site-directed mutagenesis. These degenerate pools of oligonucleotides contain a mixture of normal and abnormal bases at each position in the sequence at which a mutagenic event is desired. In the remainder of this section, we present guidelines for ways in which these populations of clustered mutations can be efficiently generated using degenerate pools of mutagenic oligonucleotides.

Use of Degenerate Pools of Mutagenic Oligonucleotides

1. Pools of degenerate single-stranded oligonucleotides can be used only when the target amino acids are clustered. If all of the codons that are to be altered lie within a short stretch of contiguous nucleotides, a pool of degenerate single-stranded mutagenic oligonucleotides can be used as mismatched primers on single-stranded DNA templates to generate the corresponding set of mutants. However, the mutants cannot generally be distinguished from the original wild-type DNA by the standard method of screening by hybridization. In most cases, the mutagenic oligonucleotides are so long that there is no practical difference in stability between mismatched and perfect hybrids. Even if the oligonucleotides are sufficiently short (≤ 20 nucleotides in length), the pool usually contains many different members, each of which has different hybridization characteristics. It is therefore extremely difficult, if not impossible, to devise hybridization conditions that will distinguish all possible mutant sequences from the original wild-type sequences. This type of mutagenesis is therefore best carried out using the Kunkel system (see pages 15.74–15.79), which selects strongly against bacteriophages generated by replication of the original wild-type (+) strand of DNA. Mutants are then identified by picking individual plaques blindly and sequencing the relevant section of single-stranded bacteriophage DNA.

2. An alternative method is to generate pools of mutants by "cassette mutagenesis," a technique that involves replacing the wild-type sequence with synthetic double-stranded oligonucleotides (see, e.g., McNeil and Smith 1985; Wells et al. 1985; Derbyshire et al. 1986; Hill et al. 1986, 1987; Hutchison et al. 1986; Bedwell et al. 1989). Since cassette mutagenesis was first introduced (Matteucci and Heyneker 1983), several variations have been described, each of which has advantages under particular circumstances. However, all of these techniques suffer from a common drawback—the necessity for unique restriction sites at both ends of the cassette. Because these restriction sites are required to shuttle the synthetic double-stranded oligonucleotide into the correct location, they cannot occur anywhere else in either the plasmid vector or the segment of the wild-type gene that it carries. Furthermore, to ensure that the cassette is inserted in the correct orientation, the cassette should carry different restriction sites at each end. Because naturally occurring restriction sites hardly ever fulfill these criteria, it is usually necessary to carry out one or more rounds of site-directed mutagenesis to create suitable restriction sites at the appropriate locations in the wild-type gene. If the introduction of these sites changes the amino acid sequence encoded by the gene, it is necessary to determine whether the resulting protein displays wild-type characteristics. To eliminate the possibility that the phenotypes of any mutants obtained by cassette mutagenesis result from a combination of amino acid changes (i.e., changes caused by introduction of the restriction sites and by changes encoded within the cassette), it may be necessary to restore the original wild-type sequence at the restriction sites.

   Three different methods are currently used to generate double-stranded

oligonucleotide cassettes. In the first method (McNeil and Smith 1985) (see Figure 15.9A), two separate sets of oligonucleotides are synthesized that are complementary to the opposite strands of the target DNA. One of these sets consists of a single species of oligonucleotide that is exactly complementary to the sequence of one of the strands of the wild-type target DNA. The other set consists of a degenerate pool of oligonucleotides that is complementary to the opposite strand and that carries the desired mutations. These sets of complementary oligonucleotides are then mixed under conditions that will allow mismatched hybrids to form. If the complementary oligonucleotides have been designed to yield double-stranded cassettes that carry the appropriate protruding termini, they can be inserted directly into a recombinant plasmid in place of the homologous wild-type sequence. Alternatively, cohesive termini can be created by digesting double-stranded blunt-ended cassettes with the appropriate restriction enzymes. The mismatches in the recombinant plasmids are repaired in vivo, after the recombinant plasmids have been introduced into competent bacteria. Subsequent replication of the plasmid DNA and segregation into daughter cells allows clones to be isolated that are derived from each DNA strand of the plasmid originally used for transformation. In this method and the one that follows, the plasmids isolated from individual colonies of transformed bacteria are occasionally heterogeneous, suggesting that segregation of the plasmids is sometimes incomplete. This problem can be solved by retransforming competent bacteria with plasmid DNAs extracted from pooled primary transformants. However, in this first method of cassette mutagenesis, the frequency of mutation can never exceed 50% because only one half of the progeny plasmids are derived from the mutagenized strand.

In the second method (see Figure 15.9B), the frequency of mutation is increased by using cassettes in which the complementary strands both consist of mixed-sequence oligonucleotides (Wells et al. 1985). Because each of these strands gives rise to progeny plasmids, the mutation rate can be raised to greater than 50% (Makris et al. 1988).

In the third method (see Figure 15.9C), degenerate pools of single-stranded oligonucleotides are converted to a blunt-ended double-stranded form by mutually primed synthesis (Oliphant et al. 1986; Hill et al. 1987). Two degenerate pools of oligonucleotides are synthesized that are complementary to the same strand of the target DNA. However, the members of one pool carry sequences at their 3′ termini that are complementary to sequences at the 3′ termini of oligonucleotides in the second pool. Usually, these complementary sequences are palindromic and correspond to the restriction site that marks one end of the cassette. The oligonucleotides in the two pools are then annealed to form partial hybrids that can be converted to blunt-ended double-stranded DNA by the Klenow fragment of *E. coli* DNA polymerase I. The products of this reaction are tail-to-tail dimers. Unit-length cassettes are generated by digesting the dimers with the appropriate restriction enzymes.

The major advantage of the third method is that the unit-length cassette consists of perfect homoduplexes. Any potential bias that occurs during mismatch repair in vivo is therefore avoided, and there is no loss of

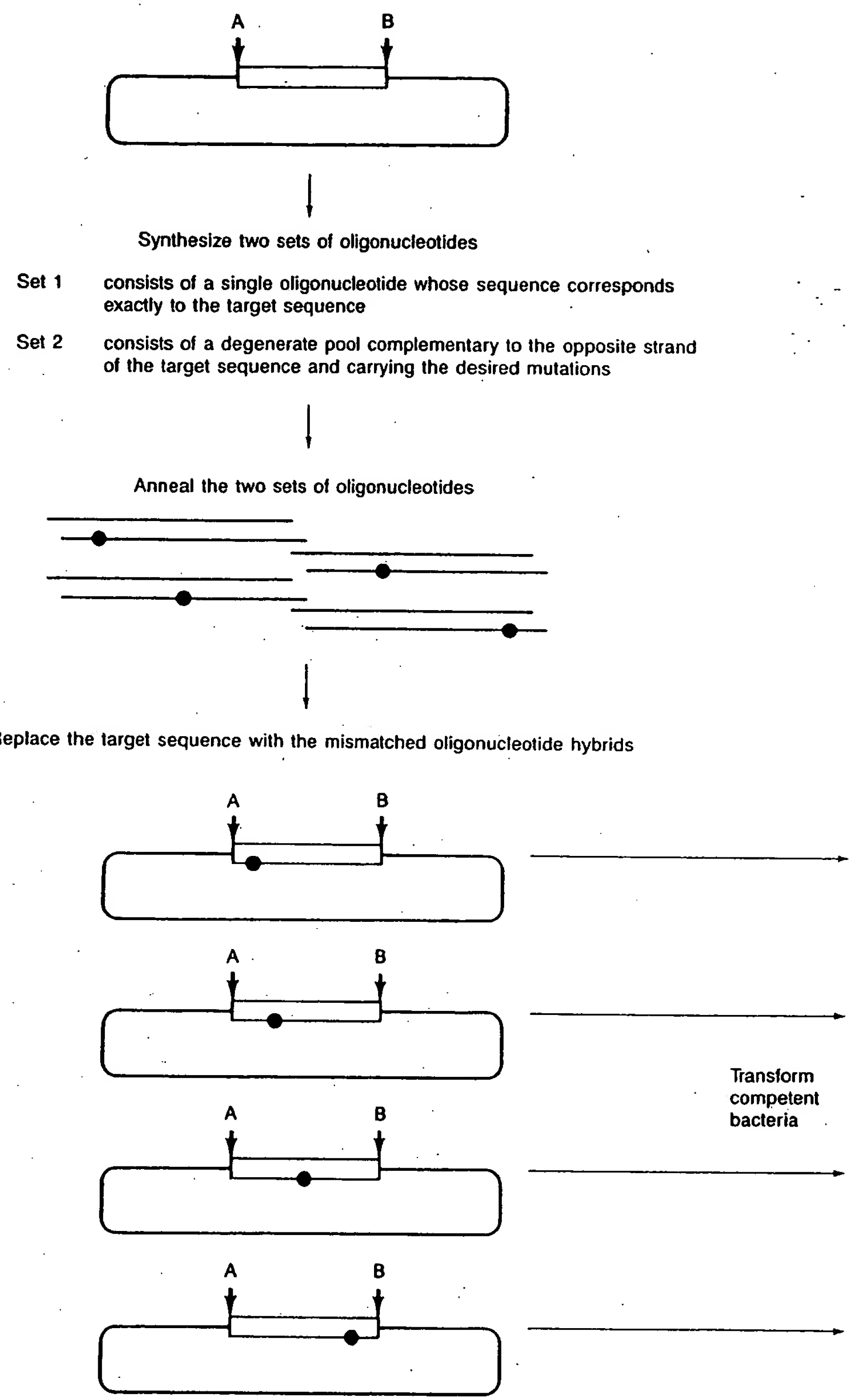
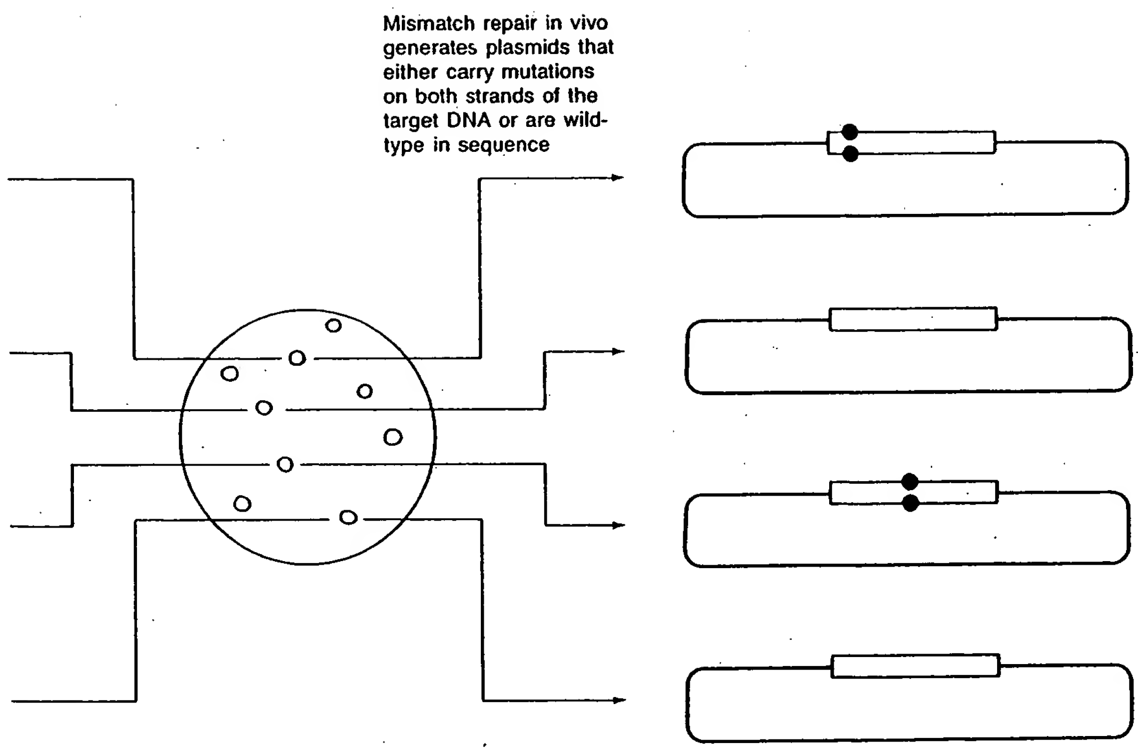


FIGURE 15.9A
Cassette mutagenesis using a single mixed-sequence oligonucleotide and repair of mismatches in vivo.


Mismatch repair in vivo generates plasmids that either carry mutations on both strands of the target DNA or are wild-type in sequence

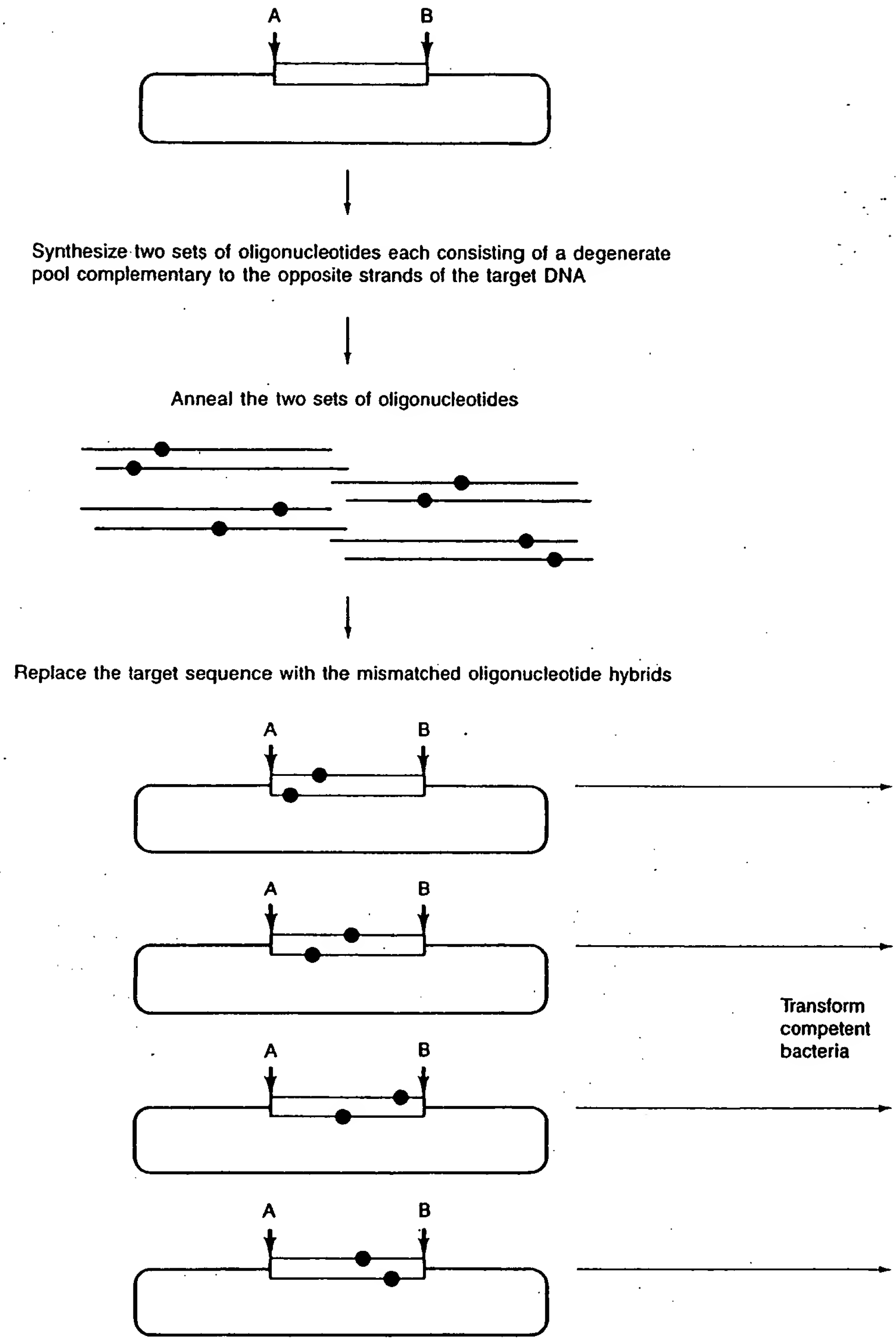
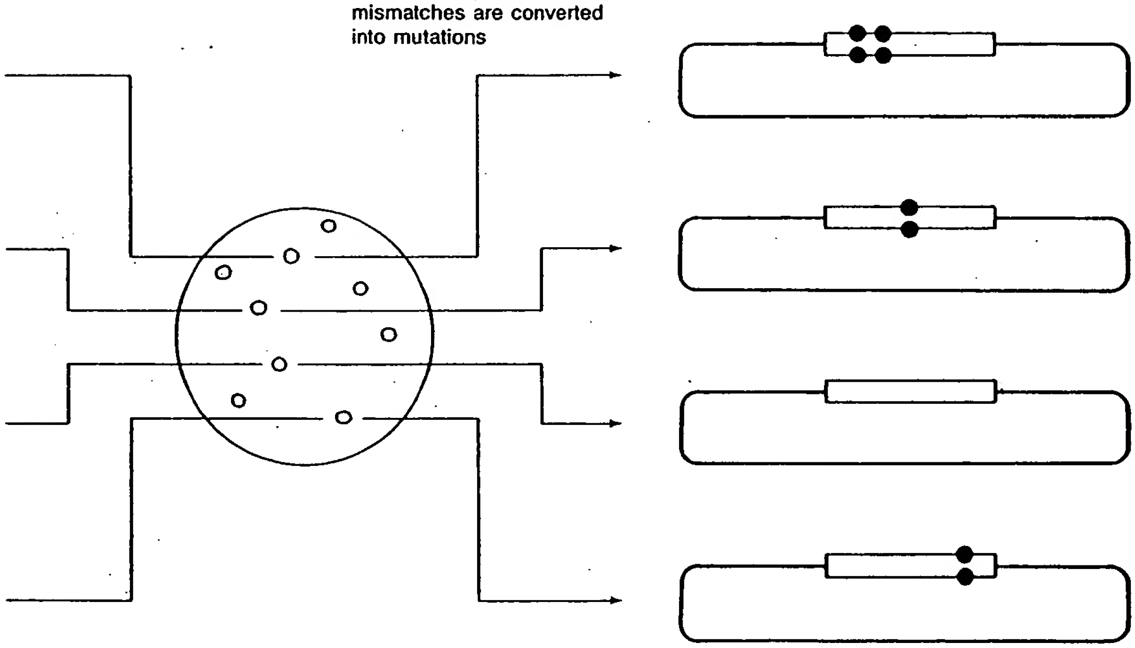


FIGURE 15.9B

Cassette mutagenesis using two complementary mixed-sequence oligonucleotides and repair of mismatches in vivo.

Mismatched repair in vivo
generates plasmids in which
approximately 50% of the
mismatches are converted
into mutations



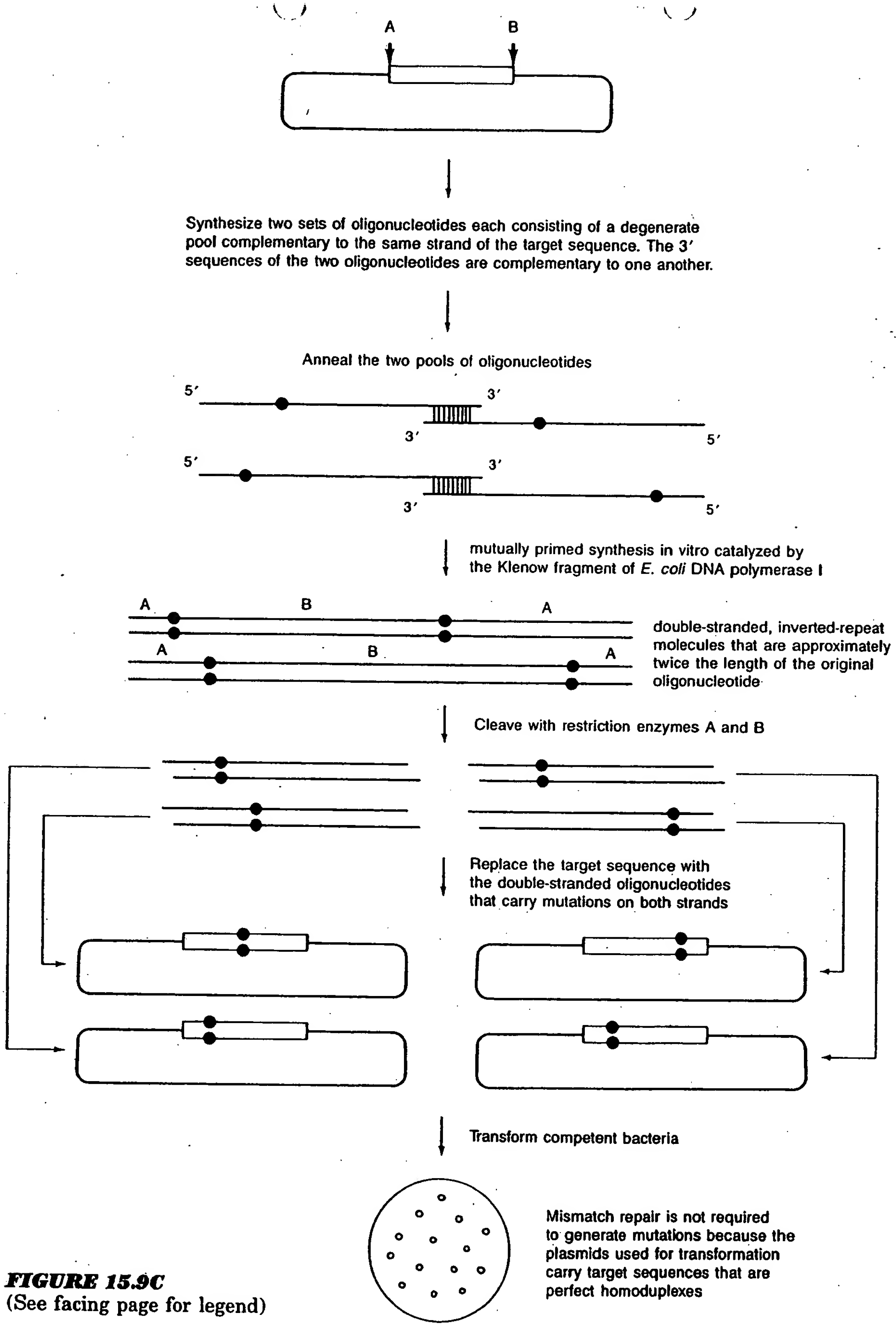


FIGURE 15.9C
(See facing page for legend)

mutants because of correction to wild-type sequences. Finally, because segregation is not required, primary bacterial transformants contain pure plasmid populations that can be analyzed directly. For these reasons, this is currently the method of choice for creating mutations at many sites within a defined region of DNA.

3. Ideally, each member of a degenerate pool of oligonucleotides should contain one nucleotide change per target sequence. In practice, however, because the oligonucleotide pools are generated in a mixed synthetic reaction, the best that can be achieved is an *average* of one altered base per target sequence. At each cycle, therefore, there is a chance that either a normal or an altered base will be incorporated into a growing oligonucleotide chain. The mutation frequency at any given site depends on the relative concentrations of the different nucleotide precursors that are provided at a particular cycle in the synthetic reaction.

   The fraction of oligonucleotides that contain nucleotide changes follows a binomial distribution that can be predicted from the following equation (McNeil and Smith 1985; Makris et al. 1988):

   $$F(P) = n!P^{n-r}(1-P)^r/(n-r)!r!$$

   where $F(P)$ is the fraction of the population whose sequence contains r random base changes over a target sequence of n consecutive bases, P is the probability of any given nucleotide being unchanged, and $(1-P)$ is the probability of any given nucleotide being changed. For example, when the length of the target sequence is 20 and the mixture of precursors supplied at every cycle contains 95% of the "normal" nucleotides and 5% of the "altered" nucleotides, the fraction of oligonucleotides that contain one altered nucleotide ($r = 1$) can be calculated as follows:

   $$\begin{aligned} F(P) &= 20!(0.95)^{19}(0.05)^1/19!1! \\ &= 0.38 \end{aligned}$$

   Similarly, 36% of the oligonucleotides in the pool will contain no alteration in nucleotide sequence; 19% of the oligonucleotides will contain two changes, and 7% will contain more than two changes.

4. The types of mutations created by degenerate pools of oligonucleotides depend on the precursors that are supplied at each round of the synthetic cycle. In the example discussed above, not more than 5% of the precursors provided at each round of synthesis can contain "abnormal" nucleotides. Within this 5%, however, the ratio of the three abnormal bases can be altered according to the needs of the particular experiment. Because transversions are usually more useful than transitions, many workers increase the proportion of abnormal bases that will generate transversions at the expense of abnormal bases that will cause transitions.

5. The termini of the oligonucleotides should not be mutagenized because they will be needed to insert the cassette into the appropriate plasmid. If

FIGURE 15.9C
Cassette mutagenesis using two partially overlapping mixed-sequence oligonucleotides and complementary strand synthesis in vitro.

cohesive termini are to be generated by cleaving the double-stranded cassettes with restriction enzymes, three extra nucleotides should be added to each end of the mutagenic oligonucleotide. These extensions increase the efficiency of digestion with restriction enzymes.

6. The frequency with which mutants are obtained at any particular position decreases as the length of the mutagenic oligonucleotides in the degenerate pool increases. Because individual mutants are recovered by random sampling, it is improbable that all possible mutations will be isolated when the size of the potential pool is large. Under these circumstances, "missing" clones that carry particularly interesting mutations can usually be identified by hybridization to specifically designed oligonucleotide probes.

Finally, it is worth remembering that oligonucleotide-mediated mutagenesis is not the only method that can be used to saturate segments of cloned DNA with mutations. Several of the other techniques that are available are discussed below.

Treatment of Double-stranded DNA with Chemical Mutagens

The simplest method of localized random mutagenesis is to react a short fragment of double-stranded DNA with a chemical mutagen such as nitrous acid or hydroxylamine and to clone the population of mutagenized fragments into a recombinant plasmid that carries the remainder of the wild-type gene. Recombinant plasmids carrying mutations that generate a novel phenotype can be recognized by appropriate tests. For example, a temperature-sensitive mutation constructed in a gene coding for a mammalian protein might be recognized by immunofluorescent staining of mammalian cells that had been transfected with the appropriate plasmid incubated at permissive and non-permissive temperatures. Recombinant plasmids carrying a mutation that does not give rise to an easily assayed phenotype must be identified by DNA sequencing of random clones. Unfortunately, the frequency at which mutants are recovered by this method is unacceptedly low (Chu et al. 1979; Solnick 1981; Busby et al. 1982; Kadonaga and Knowles 1985). Furthermore, because chemical mutagens react with bases in double-stranded DNA in a highly specific manner, only a limited spectrum of mutations is recovered. For these reasons, this method is no longer in widespread use.

Treatment of Single-stranded DNA with Sodium Bisulfite

In the original descriptions of this protocol, circular double-stranded plasmid DNA was nicked at a random site with pancreatic DNAase I in the presence of ethidium bromide (Greenfield et al. 1975; Shortle and Botstein 1983). The nick was then converted to a gap by digestion with exonuclease III, and the resulting gapped double-stranded molecule was exposed at slightly acid pH to sodium bisulfite (1–3 M), which caused deamination of cytosine to uracil. After transformation of bacteria, replication of the mutagenized DNA led to replacement of the original C:G base pair with a T:A base pair. Recently, the efficiency of this type of mutagenesis has been improved by carrying out deamination on gapped duplexes of bacteriophage M13 recombinants in which the target DNA is exposed in a single-stranded form (Pine and Huang 1987). After mutagenesis, the DNA is transfected into an *ung*$^-$ strain of *E. coli* that is unable to remove the newly generated uracil residues. Although the procedure results in highly efficient mutagenesis of a defined segment of DNA, it generates only transition mutations in which a purine replaces a purine on one strand of DNA and a pyrimidine replaces a pyrimidine on the other. Unfortunately, mutations of this type generally result in conservative substitutions of amino acids. Thus, the range of mutants that are obtained is often too narrow to allow a comprehensive analysis of a particular segment of a protein (Shortle and Nathans 1978; DiMaio and Nathans 1980; Peden and Nathans 1982).

Treatment of Single-stranded DNA with Chemicals That Damage All Four Bases

In this method (Myers et al. 1985a), single-stranded DNA of a recombinant M13 bacteriophage is exposed under defined conditions to chemicals (nitrous acid, formic acid, and hydrazine) that modify bases in single-stranded DNA without breaking the phosphodiester backbone (see Chapter 13). After removal of the chemicals, a universal sequencing primer and avian reverse transcriptase are used to synthesize the complementary strand of DNA. When the polymerase encounters damaged bases in the template strand, it incorporates nucleotides essentially at random. Because all possible nucleotides can be incorporated at a single position, there is a 75% probability of mutation at every site of damage. Furthermore, because tranversions are generated twice as frequently as transitions, the resulting mutations generate proteins with a wide spectrum of amino acid changes. After the extension reaction is completed, the double-stranded target fragment is excised and recloned into an appropriate vector. Mutants can be identified directly by DNA sequencing of random clones.

The major problem with this method is the frequency with which useful mutations can be isolated. To prevent the formation of unacceptable numbers of multiple mutants, it is necessary to limit carefully the length of time the single-stranded DNA is exposed to damaging chemicals. However, this means that many of the template strands escape modification altogether. Therefore, the best that can be achieved by this method is a frequency of single mutations of 10–15%. This problem can sometimes be alleviated by using denaturing gradient gel electrophoresis to purify fragments of DNA that carry mutations (Myers et al. 1985a,b). However, this technique is by no means simple, and it requires the attachment of the mutagenized DNA to special vectors equipped with GC clamps (Myers et al. 1985c). Because of these problems, this method of mutagenesis has so far not found widespread acceptance.

Point mutations can be introduced into double-stranded DNA by incorporating base analogs with various types of DNA polymerases. For example, Shortle and his coworkers (Shortle et al. 1982; Shortle and Lin 1985) incubated gapped DNA in the presence of *E. coli* DNA polymerase I and only one of the four α-thiophosphate dNTPs. Thiophosphate dNTPs are efficiently incorporated by the polymerase but are not effectively removed by its $3' \rightarrow 5'$ editing function. The incorrect base is thus incorporated at a high frequency, and the remainder of the gap is then filled in a second polymerization reaction carried out in the presence of all four of the normal dNTPs. All types of base substitutions have been obtained with this method using each of the four α-thiophosphate dNTPs in separate repair reactions.

Another misincorporation method uses AMV reverse transcriptase, which is deficient in a $3' \rightarrow 5'$ exonuclease activity (Zakour and Loeb 1982). In this case, conventional dNTPs are used to synthesize DNA from an upstream primer. Base analogs are then incorporated in the region of interest.

The major problem of these and other misincorporation methods is the difficulty in creating populations of template molecules in which the 3' hydroxyl terminus of the growing strand is located at random positions throughout the region of interest. Although this can in theory be achieved by a number of different methods (e.g., controlled nick translation with *E. coli* DNA polymerase I or controlled digestion of double-stranded DNA with exonuclease III), the routine generation of large numbers of mutations at random sites has proved to be difficult in practice. Success requires careful characterization of the reagents involved, meticulous establishment of optimal reaction conditions, and many trial experiments.

In summary, whereas methods (discussed earlier in this chapter) to introduce single mutations in cloned DNA are now well-established, techniques to saturate defined regions with mutations are less satisfactory. Using chemical mutagenesis, the rate of production of single mutations is low and/or the mutations themselves are of limited interest. Using misincorporation of base analogs, it is difficult to direct the mutations to the region of interest. However, it seems likely that at least some of these problems will be solved during the next few years, for example, by incorporating base changes into DNA synthesized in polymerase chain reactions or by advances in DNA chemistry that will facilitate the synthesis of mutagenized DNAs of extended length. Until then, we recommend using degenerate pools of synthetic oligonucleotides. In contrast to the other methods, the mutations can be precisely designed by the experimenter and can be focused in a defined region of DNA. Even with these limitations, the amount of work involved in isolating and characterizing a comprehensive set of mutants remains very large. In this branch of molecular cloning, therefore, it is especially important to weigh the potential scientific rewards against the commitment of time and personnel that the project will certainly consume.